

FORM-PTO-1360
(Rev. 9-2001)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

033493-001

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)

10/009643INTERNATIONAL APPLICATION NO.
US00/16135INTERNATIONAL FILING DATE
June 12, 2000PRIORITY DATE CLAIMED
~~December 12, 2001~~ 6-12-99

TITLE OF INVENTION

CHICKEN GROWTH HORMONE RELEASING HORMONE RECEPTOR

APPLICANT(S) FOR DO/EO/US
MICHAEL O. THORNER

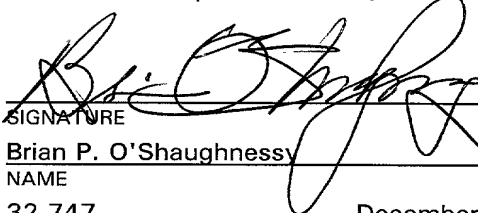
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto.
 - b. ☒ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☒ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information:

**21839**

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 10/009643		INTERNATIONAL APPLICATION NO. US00/16135		ATTORNEY'S DOCKET NUMBER 033493-001	
21. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,040.00 (960) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 (970) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 (958) International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 (956) International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962)					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	17 -20 =	-0-	X\$18.00 (966)	\$	
Independent Claims	15 -3 =	12	X\$84.00 (964)	\$1,008.00	
Multiple dependent claim(s) (if applicable)			+ \$280.00 (968)	\$	
TOTAL OF ABOVE CALCULATIONS =				\$1,718.00	
Reduction for 1/2 for filing by small entity, if applicable (see below). +				\$859.00	-
SUBTOTAL =				\$859.00	
Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$	
TOTAL NATIONAL FEE =				\$859.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$	
TOTAL FEES ENCLOSED =				\$859.00	
				Amount to be refunded:	\$
				charged:	\$
a. <input checked="" type="checkbox"/> Small entity status is hereby claimed. b. <input checked="" type="checkbox"/> A check in the amount of \$ <u>859.00</u> to cover the above fees is enclosed. c. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. d. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Brian P. O'Shaughnessy, Esq. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620					
			 SIGNATURE Brian P. O'Shaughnessy NAME	32,747 REGISTRATION NUMBER	
			December 12, 2001 DATE		

Patent
Attorney's Docket No. 033493-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Thorner et al)	Group Art Unit: Unassigned
)	
Application No.: TBA (§371 of US00/16135))	Examiner: Unassigned
)	
Filed: December 12, 2001)	
)	
For: CHICKEN GROWTH HORMONE)	
RELEASING HORMONE)	
RECEPTOR)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the referenced application, please interpose the following amendments to the claims in the referenced application.

IN THE CLAIMS:

Please re-number the following claims as follows:

15. A nucleic acid sequence encoding the chicken GHRH receptor, said sequence comprising the nucleic acid sequence of SEQ ID NO: 3.
16. A transgenic avian species comprising a nucleic acid sequence encoding the protein of claim 3.
17. A transgenic avian species comprising a nucleic acid sequence encoding the protein of claim 7.

REMARKS

Due to a typographical error, the claims as originally filed and those as published in the corresponding PCT application contained no claim numbered 15. By this amendment, Applicants are amending the numbering of the claims to correct that error.

If, at any time, the Examiner has any questions or comments regarding the structure or content of the attached application, the Examiner is encouraged to call Applicants' representative at the number provided below. It is submitted that such informal communication will expedite examination.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: 

Brian P. O'Shaughnessy
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Date: December 12, 2001

Attachment to Preliminary Amendment dated December 12, 2001

Marked-up Claims 16-18

15. [16] A nucleic acid sequence encoding the chicken GHRH receptor, said sequence comprising the nucleic acid sequence of SEQ ID NO: 3.

16. [17] A transgenic avian species comprising a nucleic acid sequence encoding the protein of claim 3.

17. [18] A transgenic avian species comprising a nucleic acid sequence encoding the protein of claim 7.

3/pst

Chicken Growth Hormone Releasing Hormone Receptor

Field of the Invention

The present invention is directed to chicken growth hormone releasing
5 hormone GHRH receptor, ligands that bind to the GHRH receptor, and nucleic acid
sequences encoding the GHRH receptor.

Background of the Invention

Growth hormone releasing hormone (GHRH) is a hypothalamic
10 hormone that acts at a pituitary receptor to stimulate the pulsatile release of GH. In
mammals, GH is required for normal growth and development in the young, and has
continuing importance in adults affecting such diverse functions as muscle
maintenance, fat deposition, skin thickness, wound healing and exercise performance.
GHRH also has direct effects on sleep. These actions have widespread clinical
15 implications not only when considering GH deficiency syndromes, but also the
sharply diminished GH levels that occur with aging and obesity. The anabolic and
anticatabolic activities of GH have also recently been shown to ameliorate the muscle
wasting and weight loss seen with AIDS.

GHRH and other regulators of the GH axis also have great potential in
20 agricultural applications because they can stimulate growth and improve the efficiency
of feed utilization. They control the relative partitioning of nutrients between muscle
and fat and so may allow the production of leaner livestock and higher yields of milk,
hair and feathers.

In birds, the function and regulation of GH are not well understood and
25 thyroid releasing hormone (TRH) as well as gonadotropin releasing hormone (GnRH)
appear to be a major factors in GH release. Research studies have been inconclusive
as to the physiological role of GHRH in birds. A putative chicken GHRH polypeptide
has been cloned and synthesized but found to have little or no GH releasing activity at
chicken pituitary cells or in live chickens. Despite this, chicken pituitaries and live
30 chickens do respond to mammalian GHRH with GH release. Studies in chickens
using mammalian GHRH have not been successful in improving growth. Thus, it has
been speculated that chickens have no functional GHRH receptor.

Furthermore, Southern blot analysis, using a human GHRH receptor probe and genomic DNA from human, monkey, rat, mouse, dog, cow, rabbit, chicken and yeast, detected GHRH receptors in all mammals tested, but not in chicken or yeast. These results indicate that GHRH receptors are well conserved in all the mammals tested, but absent or less well conserved in the chicken. Binding of human GHRH to chicken pituitary membranes suggests a high affinity G protein coupled receptor for GHRH is present in chicken. A similar experiment using the putative chicken GHRH and chicken pituitary membranes detected no specific binding. Thus the function of GHRH in birds is not understood and this prevents the development of its agricultural applications.

Further investigation of GHRH's role in avian development required the isolation of the corresponding GHRH receptor (GHRH-R). Purification of pituitary receptors is very difficult because of the scarcity of tissue, problems involving the solubilization of the receptors in active form, and in developing an efficient purification method. Therefore a need exists for the isolation of the gene that encodes the GHRH-R (or biologically active fragments thereof) to allow for the large scale production of GHRH-R. There is also a need for a vector, host cell, or host organisms comprising a nucleic acid sequence encoding protein or polypeptides having the activity of GHRH-R.

Large scale production of the cloned chicken GHRH receptor would enable the screening of large numbers of GHRH analogs for identification of improved agonists and antagonists. Such agonists and antagonists will have utility in improving feed utilization and enhancing the efficient production of larger, leaner chickens and other avian species used for meat production.

Summary of the Invention

The chicken GHRH receptor has been cloned and functions similar to the natural source tissue (chicken pituitary cells) in that it responding to human GHRH but not to the reported chicken hormone. This led us to suspect a problem with the supposed chicken GHRH hormone. The chicken GHRH polypeptide was then recloned from freshly flash frozen chicken hypothalami and sequenced. The nucleic acid sequence of the newly isolated chicken GHRH gene did not agree with

the published sequence and the synthesized chicken hormone based on this new sequence was found to be active at the cloned chicken GHRH receptor. The present invention is directed to the use of the new chicken GHRH hormone and its corresponding GHRH receptor to enhance the production of larger, leaner chickens and other avian species used for meat production.

Brief Description of the Drawings

Fig. 1A and 1B is a graph showing the binding activity of the reported chicken GHRH (Fig. 1A) and the present chicken GHRH (Fig. 1B) at the recombinant chicken GHRH receptor. Binding at recombinant chicken GHRH receptor is competed by the new chicken polypeptide (Fig 1B) but not by the published polypeptide (Fig 1A).

Figs. 2A and 2B is a graph showing cAMP signaling through the recombinant chicken GHRH receptor. Fig. 2A shows that the published polypeptide is inactive in stimulating second messenger signaling at the cloned chicken GHRH receptor, while Fig. 2B shows that the cloned chicken GHRH polypeptide reported herein is active.

Fig. 3 Binding activity at chicken pituitary membranes, showing that the new polypeptide sequence is active at the endogenous receptor in chicken pituitaries.

Detailed Description of the Invention

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, "nucleic acid," "DNA," and similar terms also include nucleic acid analogs, i.e. analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention.

As used herein, the term "purified" means that the molecule or compound is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment.

The invention also encompasses nucleic acid molecules and polypeptides which differ from actual nucleic acid and polypeptide molecules shown in the Sequence Listing, but which produce the same phenotypic effect. These

altered, but phenotypically equivalent nucleic acid and polypeptide molecules are referred to as "equivalent nucleic acids" and "equivalent polypeptides", respectively. This invention also encompasses nucleic acid molecules characterized by changes in non-coding regions that do not alter the phenotype of the polypeptide produced therefrom when compared to the nucleic acid molecule of the present invention. As used herein, the term "nucleic acid" encompasses RNA as well as single and double-stranded DNA and cDNA.

The term "peptide" encompasses a sequence of 3 or more but less than 16 amino acids wherein the amino acids are naturally occurring or synthetic (non-naturally occurring) amino acids. The term polypeptide as used herein is a sequence of 16 or more amino acids wherein the amino acids are naturally occurring or synthetic (non-naturally occurring) amino acids. Peptide or polypeptide mimetics include peptides or polypeptides having one or more of the following modifications:

1. sequences wherein one or more of the peptidyl --C(O)NR-- linkages (bonds) have been replaced by a non-peptidyl linkage such as a --CH₂-carbamate linkage (---CH₂OC(O)NR--), a phosphonate linkage, a -CH₂-sulfonamide (-CH₂-S(O)₂NR--) linkage, a urea (--NHC(O)NH--) linkage, a --CH₂-secondary amine linkage, or with an alkylated peptidyl linkage (--C(O)NR--) wherein R is C₁-C₄ alkyl;
2. sequences wherein the N-terminus is derivatized to a --NRR₁ group, to a --NRC(O)R group, to a --NRC(O)OR group, to a --NRS(O)₂R group, to a --NHC(O)NHR group where R and R₁ are hydrogen or C₁-C₄ alkyl with the proviso that R and R₁ are not both hydrogen;
3. sequences wherein the C terminus is derivatized to --C(O)R₂ where R₂ is selected from the group consisting of C₁-C₄ alkoxy, and --NR₃R₄ where R₃ and R₄ are independently selected from the group consisting of hydrogen and C₁-C₄ alkyl.

Naturally occurring amino acid residues in peptides/polypeptides are abbreviated as recommended by the IUPAC-IUB Biochemical Nomenclature

Commission as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Norleucine is Nle; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gln or Q; Asparagine is Asn or N; Lysine is

Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; Glycine is Gly or G, and X is any amino acid. Other naturally occurring amino acids include, by way of example, 4-hydroxyproline, 5-hydroxylysine, and the like.

5 Synthetic or non-naturally occurring amino acids refer to amino acids which do not naturally occur *in vivo* but which, nevertheless, can be incorporated into the peptide/polypeptide structures described herein. The resulting "synthetic peptide" contain amino acids other than the 20 naturally occurring, genetically encoded amino acids at one, two, or more positions of the peptides. For instance, naphthylalanine can
10 be substituted for tryptophan to facilitate synthesis. Other synthetic amino acids that can be substituted into peptides include L-hydroxypropyl, L-3,4-dihydroxyphenylalanyl, alpha-amino acids such as L-alpha-hydroxylysyl and D-alpha-methylalanyl, L-alpha.-methylalanyl, beta.-amino acids, and isoquinolyl. D amino acids and non-naturally occurring synthetic amino acids can also be
15 incorporated into the peptides. Other derivatives include replacement of the naturally occurring side chains of the 20 genetically encoded amino acids (or any L or D amino acid) with other side chains.

As used herein, the term "conservative amino acid substitution" are defined herein as exchanges within one of the following five groups:

20 I. Small aliphatic, nonpolar or slightly polar residues:

Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides:

Asp, Asn, Glu, Gln;

III. Polar, positively charged residues:

25 His, Arg, Lys;

IV. Large, aliphatic, nonpolar residues:

Met Leu, Ile, Val, Cys

V. Large, aromatic residues:

Phe, Tyr, Trp

30 As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil

emulsion, and various types of wetting agents.

As used herein, the term "biologically active fragments" of the GHRH receptor encompasses natural or synthetic portions of the full-length receptor which are capable of binding a receptor-specific ligand (i.e. GHRH polypeptide), or which
5 are capable of eliciting in a host animal GHRH-R specific antisera or a second messenger response while either conjugated to a carrier or in nonconjugated form.

In mammals growth hormone releasing hormone (GHRH) stimulates GH release and synthesis from the somatotropes of the anterior pituitary. The GHRH
10 receptor is a member of family B of the seven transmembrane, G-protein coupled receptors. In the chicken, GHRH polypeptides from mammals and fish stimulate GH release from the pituitary *in vivo* and *in vitro* suggesting the presence of GHRH like receptors on the chicken somatotropes, however, the recently cloned and synthesized
15 chicken GHRH is a very poor GH secretagogue. In addition a specific GHRH receptor has not been characterized in the chicken, raising questions about the regulation of GH secretion. A zoo blot probed with a 1kb fragment of human GHRH receptor DNA failed to elicit a signal in the chicken. This raises questions about the regulation of GH secretion in the chicken.

As described herein the chicken GHRH has now been isolated and
20 used to identify a new chicken GHRH that is capable of activating the chicken GHRH receptor. The identification of the chicken GHRH receptor implies that GH secretion is under the control of regulatory mechanisms similar to those in mammals and will allow more detailed study of this endocrine system in chickens. It will also contribute to structure/function studies of how the GHRH receptor works by indicating receptor
25 domains that are required to be conserved and those that are important for ligand specificity. The chicken GHRH receptor will also be used in accordance with the present invention to identify GHRH analogs and mimetics that stimulate GH release in poultry. This will have great use agriculturally for the improvement of feed utilization and the efficient production of larger, leaner chickens and other
30 commercially viable avian species.

The present invention is directed to the use of the chicken GHRH polypeptide and the chicken GHRH receptor to enhance the production of larger,

leaner chickens and other avian species used for meat production and enhance feed utilization. In accordance with one embodiment, a chicken GHRH polypeptide, comprising the amino acid sequence of SEQ ID NO: 1 or a polypeptide that differs from SEQ ID NO: B by one or more conservative amino acid substitutions yet retains its ability to stimulating second messenger signaling at the cloned chicken GHRH receptor, is administered to an agriculturally significant avian species to enhance the growth of the avian species.

The sequence of a chicken GHRH polypeptide has been previously reported however that polypeptide has been tested in chickens and chicken pituitary cells, and had little or no GH releasing activity while human GHRH was found to be active. The present invention is directed to GHRH polypeptides that are capable of stimulating second messenger signaling at the chicken GHRH receptor comprising the sequence of SEQ ID NO: 4. The sequence of the chicken GHRH gene, with the 5' and 3' untranslated region (start codon located at bp 54, stop codon located at bp 1312) is shown as SEQ ID NO: 1. The coding sequence of chicken GHRH is shown as SEQ ID NO: 6. In accordance with one embodiment of the present invention a chicken GHRH having the amino acid sequence of SEQ ID NO: 2 is provided. This sequence differs from the previously reported sequence at amino acid number 21, wherein the present sequence has a lysine and the previously reported sequence has an asparagine.

Synthesis of the chicken GHRH(1-33)NH₂, based on the nucleotide sequence of SEQ ID NO: 1, produced a polypeptide that is active in binding to chicken pituitary membranes and competes with human GHRH. At the recombinant chicken GHRH receptor the new polypeptide is active in binding and in signaling through the second messenger cAMP. This new chicken polypeptide is thus a functional GHRH. While the new sequence is active, it is less potent at the chicken receptor than human GHRH. This may be inherent to chicken physiology, or it may be an artifact of the truncated polypeptide (1-33) was tested. Only amino acids 1-29 are required for full GHRH activity in mammalian systems that have been tested. This may be different in other animals. Active fragments of the chicken GHRH are also within the scope of this invention, including but not limited to truncated polypeptides that include amino acid residues 1-33 and 1-29 of SEQ ID NO: 2, respectively. Thus, one aspect of the present invention relates to a substantially pure

protein and biologically active fragments thereof having chicken growth hormone releasing hormone (GHRH) receptor activity.

The present invention also encompasses nucleic acid sequences that encode a peptide or polypeptide that binds to chicken GHRH receptor and is capable of signaling through the second messenger cAMP is provided. In one preferred embodiment the nucleic acid comprises the sequence of SEQ ID NO: 6. In one embodiment a transgenic avian species is provided wherein one or more of the chickens cells comprise a nucleic acid sequence encoding the chicken GHRH protein of SEQ ID NO: 2. Accordingly, the present invention provides for recombinant organisms and progeny thereof comprising an exogenous gene encoding for a chicken GHRH and biologically active fragments thereof.

The present invention is also directed to pharmaceutical formulations comprising the chicken GHRH polypeptide for administration to avian species (preferably to chickens) wherein the GHRH polypeptide interacts with the chicken GHRH receptor and produces a signal through a secondary messenger molecule. The chicken GHRH polypeptide compositions are administered to chickens to enhance feed utilization and enhance the growth and production of lean muscle mass in an avian species. In preferred embodiments the administered GHRH polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

The present invention is also directed to the GHRH receptor (GHRH-R) that binds to the GHRH polypeptide *in vivo*. The present invention provides the amino acid sequences of chicken GHRH-R and biologically active fragments thereof, as well as oligonucleotide probes or primers which can hybridize to a gene encoding chicken GHRH-R or fragments thereof. In a preferred embodiment, the invention provides for an isolated nucleic acid sequence encoding a GHRH receptor, recombinant vectors including said sequence and host cells containing said sequence useful in production of a GHRH-receptor or biologically active fragments thereof (including proteins and polypeptides having GHRH-R activity).

Preferably, a gene encoding for a protein or polypeptide having chicken GHRH-R activity is isolated and connected with a vector DNA to form a recombinant DNA; the vector DNA including said gene is capable of replicating in a prokaryotic or eukaryotic cell. The gene encoding for a protein or polypeptide having chicken

GHRH-R activity is located downstream of a promoter in the vector, and is replicated as part of the vector. The recombinant DNA is then incorporated into a host cell, which did not previously contain said gene, to form a transformed or transected cell line capable of expressing chicken GHRH-R or biologically active fragments thereof.

- 5 Accordingly, the present invention also provides for recombinant organisms and progeny thereof comprising an exogenous gene encoding for a chicken GHRH-R and biologically active fragments thereof.

The invention is also directed to pharmaceutical compositions comprising an effective amount of the pure receptor or fragments thereof, or proteins and polypeptides having chicken GHRH-R activity in combination with a
10 pharmaceutically acceptable carrier, and provides a method for the therapeutic use of such pharmaceutical compositions. The receptor and receptor fragments (proteins and polypeptides having chicken GHRH-R ligand binding or immunological activity) are useful in screening methods for identifying chicken GHRH analogs, as well as in
15 identifying compounds which may act as chicken GHRH antagonists at the receptor site. In one embodiment GHRH-R is attached to an inert substrate (such as a polymer bead) using standard techniques known to the skilled practitioner. Such bound material can be contacted with a solution of potential GHRH analogs under conditions that allow binding. The material can then be washed to allow for the removal of non-
20 specifically bound compounds, and thus identifying the remaining GHRH analogs.

In accordance with one embodiment of the present invention, a purified nucleic acid sequence is provided that encodes for the chicken growth hormone releasing hormone receptor or biologically active fragments thereof. In yet another embodiment of the present invention a vector comprising a nucleic acid
25 sequence encoding a chicken GHRH polypeptide or chicken GHRH receptor, or biologically active fragments thereof, is provided. In one embodiment the vector is an expression vector that is operably linked to nucleic acid sequences that encode the GHRH polypeptide or the GHRH receptor. Such recombinant expression vectors can be used to transform cells to produce a host cell or living cell line comprising a
30 nucleic acid sequence encoding a growth hormone releasing hormone receptor or biologically active fragment thereof. In accordance with one embodiment a transgenic avian species is produced (preferably a chicken) wherein the avian species comprises

one or more cells that express exogenously introduced recombinant GHRH polypeptide or the GHRH receptor.

It is a further object of the present invention to provide a pharmaceutical composition comprising the GHRH polypeptide of SEQ ID NO: 2, or
5 biologically active fragment thereof, and a therapeutic method for administering an effective amount of same to an organism to bind to endogenous GHRH receptor.

There is further a need for screening assays which utilize chicken GHRH-R or biologically active fragments thereof for testing compounds which may interact with chicken GHRH-R or fragments thereof. Preferably such compounds will
10 have physiological properties that allow the compounds to be administered orally. Further, it is another object of the present invention to produce recombinant chicken GHRH-R in sufficient amounts to allow large scale screening of polypeptides and xenobiotics for chicken GHRH-R receptor binding ability.

The isolated chicken GHRH receptor is also useful in raising GHRH-R
15 specific antibodies. Such antibodies may, by blocking the receptor site, effectively prevent GHRH binding and thereby block growth. Other antibodies can be used to activate the GHRH-R receptor (e.g., thyroid stimulating antibodies, such as those causing Graves Disease). Accordingly, the chicken GHRH-R, or an immunogenic portion thereof can be used to elicit antibodies which bind to the receptor and thereby
20 induce or block activity. In accordance with one embodiment, the antigenic peptide fragment of SEQ ID NO: 5 is used to elicit antibody production. Pharmaceutical compositions containing the receptor or segment fragments can be used to treat disorders resulting from or associated with an excess of circulating GHRH. Such compositions can be employed for *in vivo* administration to bind circulating GHRH,
25 thus preventing its binding to endogenous receptor.

The technique of hydrophilicity analysis of primary sequence information has been commonly used to identify both hydrophobic potentially membrane-spanning domains and hydrophilic antigenic sites. Analysis of the cloned GHRH receptor by the Hopp and Woods Method, see FIG. 18 (Hopp, T. P., and
30 Woods, K. R., Proc. Natl. Acad., 78:3824 (1981) indicates seven domains rich in hydrophobic residues; this is a common property in the G-protein linked receptor family Wang, H., Lipfert, L., Malbon, C., and Bahouth, B., J. Biol. Chem., 264:14424

(1989). This model depicts four extracellular regions which are potential targets for binding of anti-receptor antibodies; three extracellular loops (EC-1, EC-2, EC-3) and an N-terminus which contains sites for asparagine-linked glycosylation.

For purposes of producing antibodies which block or activate the
5 receptor, extracellular loop fragments, particularly those not containing N-
glycosylation sites (carbohydrate may sterically interfere with antibody binding) are preferred. However, intracellular loops (IC) are also useful in production of
antibodies which may be used for solid phase binding of the receptor protein in
screening and other assays. A recent study of antibodies directed against the three EC
10 loops of the thyrotropin receptor indicates a heterogeneity in their biological activities, including the induction of blocking antibodies using the EC-3 Loop as the antigen.
See Ohmori, M., et al., *Biochem. Biophys. Res. Comm.*, 174:399 (1991). Therefore, a
broad panel of anti-polypeptide and anti-receptor antibodies is prepared and carefully
evaluated in order to determine the epitopes required for the induction of blocking or
15 activating antibodies.

To prepare antibodies in accordance with the present invention,
polypeptides are produced by conventional solid phase synthesis cleaved by HF, and
HPLC purified (van Regenmortel, M. H., In *Synthetic Polypeptides as Antigens*,
Elsevier, New York, pages 41-93 (1988). Polypeptides are then typically coupled to a
20 carrier immunogen such as keyhole limpet haemocyanin (KLH) or ovalbumin via
glutaraldehyde by conventional procedures, and these conjugates used to immunize
mice and rabbits. See Coligan, JI et al., In *Current Protocols in Immunology*, Vol. 1,
Wiley-Interscience, New York (1991). Animals are given the first immunization of
conjugate polypeptide with Freund's Complete Adjuvant and three weeks later are
25 given weekly booster immunizations with conjugate polypeptide in Freund's
incomplete Adjuvant. Specific anti-polypeptide antibodies are detected by their
binding to polypeptide in an ELISA or RIA assay. In those instances where
polypeptides do not adhere to plastic, and therefore are not amenable for direct assay,
then the polypeptide is conjugated to an unrelated carrier protein which then adheres
30 to the plate.

The specificity of the antibodies is also evaluated by the technique of
Western Blotting. See Towbin, It. et al., *PNAS USA*, 76:4350 (1979). Specific

antibodies reorganize a 55 kDa protein in crude membrane preparations, in WGA eluted glycoproteins, or in streptavidin eluates. Membranes from a cell line devoid of receptor serve as a negative control. This procedure also gives information about the degree of cross-reactivity of the antibodies with receptor subtypes from other cell lines and tissues (brain, pituitary) and obviates the need for receptor purification from each source. It is beneficial to obtain both tissue-specific antibodies for physiological studies and cross-reactive antibodies for possible use in purification of the receptor from various tissues using immunoaffinity chromatography.

Monoclonal antibodies to the GHRH receptor are prepared using conventional methods. See Harlow, H., and Lane, D., In *Antibodies: A Laboratory Manual*, Cold Spring Harbor Lab, New York, pages 139-240 (1988). The antigens used for immunization include (1) KLH-polypeptide conjugates corresponding to the extracellular regions as described above, (2) purified receptor from chicken anterior pituitary membranes, (3) purified receptor deglycosylated with neuraminidase or N-glycosidase and repurified by SDS-PAGE and electro-elution, (4) receptor purified from recombinant sources, such as transected CHO cells, or baculovirus tested for reactivity to the polypeptide immunogen by ELISA or protein immunogen by Western blot. Those which display circulating antibodies to the receptor or its polypeptides are utilized for the preparation of hybridomas. Briefly, spleen cells are removed and fused in the presence of polyethylene glycol to SP2/D myeloma cells that are deficient in the enzyme hypoxanthine-guanine phosphoribosyl aminopterin-thymidine, which selects for true hybrids of both cell types since spleen cells do not grow in culture. Hybridoma supernates are screened for antibody to the receptor by (1) Western blots of purified receptor or WGA-eluted glycoproteins, (2) ELISA using inhibition of radiolabelled ligand binding to membranes. Those hybridomas which are positive are propagated and recloned by limiting dilution or growth in soft agar; this ensures their monoclonal nature. Positive clones are used to induce tumors in mice and accumulate the antibody in ascites fluid.

Both polyclonal and monoclonal IgG antibodies are purified by conventional ammonium sulfate precipitation and Protein A chromatography. See Harlow, supra. Antibodies are analyzed for their ability to block or activate radioligand binding to membranes in the standard binding assay described above.

Those antibodies that show promise for ligand blocking or activation are tested *in vivo* using for example a rat model, e.g., by using indwelling venous catheters on rats in order to monitor GH levels after administration of anti-GHRH receptor antibody. See Miell, J., et al., J. Endocrinol, 131:75 (1991). Those antibodies which show the most *in vivo* activity at this point are examined to define their epitopes (if not already determined) on the receptor. Those epitopes represent antigenic fragments of the receptor which when used as immunogens induce antibodies possessing the desired physiological effect of altering levels of GH production.

Example 1

Isolation of the Chicken GHRH polypeptide

RT-PCR with nested PCR primers was used to amplify the full coding region of the chicken GHRH polypeptide. The hypothalami from 10 chickens were snap frozen within two minutes of death and stored at -70°C . RNA was extracted from the hypothalami using TriReagent. The RNA was used in a reverse transcriptase (SuperScript) reaction prior to a polymerase chain reaction (Taq polymerase). The PCR products were purified by agarose gel electrophoresis and a Quiagen affinity column and then cloned into the pTarget vector (Promega) using T_4 ligase overnight at 15°C . The ligation reactions used to transform *E. coli* (JM109) were selected and screened by PCR to identify clones containing the GHRH cDNA.

Positive colonies were used to inoculate Luria broth containing ampicillin that was incubated overnight at 37°C in a shaker. The plasmid DNA was purified from the bacteria using a Quiaprep Spin Mini-Prep Kit (Quiagen). A total of nine clones from two different PCR reactions were sequenced (both forward and backward) using an automated ABI dye sequencer, and all showed lysine at position 21. The resultant cDNA sequence is shown in SEQ ID NO: , and the encoded polypeptide in SEQ ID NO: .

Example 2

Isolation of the Chicken GHRH Receptor

The anterior pituitary glands were removed from 100 chicks, snap

frozen within two minutes of death and stored at -70°C . RNA was extracted from the pituitaries using TriReagent. The bulk of the RNA was methyl mercury denatured, primed using a combination of random and oligo(dT), and used to create a size selected cDNA library in Lambda Zap II phage.

5 The remaining RNA was used to perform RT-PCR reactions utilizing degenerate primers designed to detect all known GHRH receptor cDNAs. A short segment of cDNA was sequenced confirming that we were dealing with the GHRH receptor. Specific primers were designed from this sequence which were used to screen the cDNA library for a full length receptor cDNA clone. The resultant cDNA
10 sequence for the HGRH receptor is shown in SEQ ID NO: , and the encoded polypeptide in SEQ ID NO: . (figures 3 & 4). The chicken cDNA sequence has less than 63% identity with the human GHRH receptor compared to 83 to 96% identity among known mammalian GHRH receptors.

15 **Example 3**

Competitive Binding Experiments with Chicken GHRH Receptor

 Labeled human GHRH (hGHRH) was bound to recombinant chicken GHRH receptor and the labeled GHRH was competed off with non-labeled chicken or human GHRH. Fig. 1A and 1B present the data from a competition experiment. The
20 data show chicken GHRH polypeptide (asparagine²¹) [(cGHRH(Asp²¹))] failed to compete with hGHRH (Fig. 1A) whereas chicken GHRH polypeptide (lysine²¹) [(cGHRH(Lys²¹))] did compete with hGHRH (Fig. 1B) for binding to the cGHRH receptor.

 In addition, a competition experiment utilizing chicken pituitary
25 membranes (that express the natural GHRH receptors) demonstrated that (cGHRH(Lys²¹)) bound with high affinity (i.e. by competing with bound labeled hGHRH) to the receptors, whereas the reported (asparagine²¹) chicken GHRH did not. The previously reported chicken GHRH polypeptide also had no activity in binding or signaling at the cloned recombinant chicken GHRH receptor.

30 Figures 2A and 2B show the results of GHRH mediated cAMP stimulation in HEK293 cells expressing the cGHRH receptor. Cells lacking the cGHRH receptor (HEK293) were used as the control. The data show cGHRH(Asp²¹)

is inactive in stimulating second messenger signaling at the cloned chicken GHRH receptor (Fig 2A), while Fig 2B shows that (cGHRH(Lys²¹) is active.

Example 4

5 Therapeutic Utility of the GHRH-R

The chicken GHRH-R can be used to increase growth in domestic livestock, as well as improve immune functions, appetite control, feed efficiency and nutrition. We have used RT-PCR with degenerate primers to amplify fragments of GHRH receptor cDNA from RNA prepared from flash frozen chicken pituitaries.

10 These fragments were then sequenced and used to identify a full length chicken receptor cDNA clone from a chicken pituitary cDNA library. Initial attempts using an available chicken pituitary cDNA library failed because the abundance of receptor message was too low. The available chicken pituitary libraries did not have sufficient complexity to include such rare messages.

15 In one embodiment, the chicken (*Gallus gallus*) GHRH receptor sequence is used to map the binding site of GHRH on its receptor. Mapping of the site is conducted using a series of photoaffinity crosslinking probes followed by analysis of protease cleavage maps. The differences between different species allows the identification of functional sites within receptor domains. Characterizing the
20 binding site will lead to the development of GHRH analogs and mimetics that provide pharmacological means to alter GH release.

The chicken GHRH receptor will also be used to identify GHRH analogs and mimetics that stimulate GH release in poultry. In accordance with one embodiment the nucleic acid sequence of SEQ ID NO: or SEQ ID NO: is used as a
25 probe to isolate related genes from chicken and other avian species. This could be important agriculturally for the improvement of feed utilization and the efficient production of larger, leaner chickens and other avian species used for meat production.

Claims

1. A ligand that specifically binds to the GHRH receptor of SEQ ID NO: 4.
2. A nucleic acid sequence comprising SEQ ID NO: 6.
3. A polypeptide comprising the amino acid sequence of SEQ ID NO: 2, a
5 biologically active fragment thereof, or a polypeptide that differs from SEQ ID NO: 2
by one or more conservative amino acid substitutions yet retains its ability to
stimulating second messenger signaling at the cloned chicken GHRH receptor.
4. The polypeptide of claim 3 wherein the polypeptide comprises the
amino acid sequence of SEQ ID NO: 2.
- 10 5. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5
6. The polypeptide of claim 5 wherein the polypeptide comprises the
amino acid sequence of SEQ ID NO: 4
7. A chicken GHRH receptor comprising the amino acid sequence of
SEQ ID NO: 5 or an amino acid sequence that differs from SEQ ID NO: by 1-3
15 conservative amino acid substitutions.
8. A polypeptide mimetic or protein derivative of the chicken GHRH
receptor of SEQ ID NO: 7.
9. A method of enhancing feed utilization in an avian species comprising
the step of administering a GHRH analog or derivative of said species wherein the
20 analog or derivative is an agonist or antagonist of the GHRH receptor of SEQ ID NO: 4.
10. A method of enhancing the growth and production of lean muscle mass
in an avian species comprising the step of administering a GHRH analog or
derivative to said species wherein the analog or derivative is an agonist or antagonist
of the GHRH receptor of SEQ ID NO: 4 .
- 25 11. A method of enhancing feed utilization in an avian species comprising
the step of administering a GHRH polypeptide comprising the amino acid sequence of
SEQ ID NO: 2.
12. A method of enhancing the growth and production of lean muscle mass
in an avian species comprising the step of administering a GHRH polypeptide
30 comprising the amino acid sequence of SEQ ID NO: 2.
13. A method of enhancing feed utilization in an avian species comprising
the step of administering a compound that up-regulates the expression of the GHRH

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receptor of SEQ ID NO: 4.

14. A ligand that interacts with the GHRH receptor of SEQ ID NO: 4 and stimulates second messenger signaling at the chicken GHRH receptor.

15 16. A nucleic acid sequence encoding the chicken GHRH receptor, said
5 sequence comprising the nucleic acid sequence of SEQ ID NO: 3.

16 17. A transgenic avian species comprising a nucleic acid sequence
encoding the protein of claim 3.

17 18. A transgenic avian species comprising a nucleic acid sequence
encoding the protein of claim 7.

10

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number
WO 00/76455 A2

(51) International Patent Classification⁷: **A61K**

(21) International Application Number: **PCT/US00/16135**

(22) International Filing Date: **12 June 2000 (12.06.2000)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:

60/138,768 12 June 1999 (12.06.1999) **US** ✓
60/176,387 14 January 2000 (14.01.2000) **US** ✓

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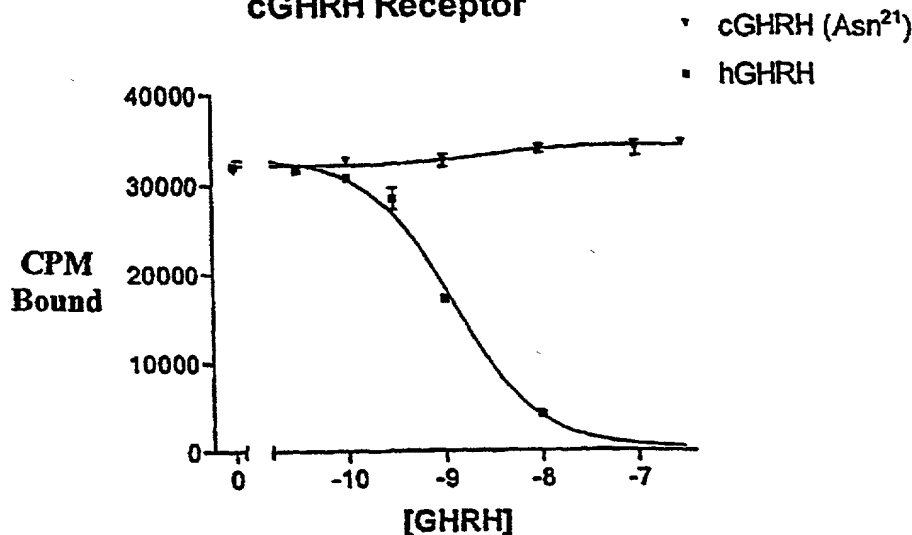
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[Continued on next page]

(54) Title: **CHICKEN GROWTH HORMONE RELEASING HORMONE RECEPTOR**

Binding at Recombinant cGHRH Receptor



(57) Abstract: The present invention relates to chicken growth hormone releasing hormone (GHRH), its corresponding receptor, and nucleic acid sequences encoding these proteins. More particularly the present invention is directed to the use of the chicken GHRH hormone and its corresponding GHRH receptor to enhance the production of larger, leaner chickens and other avian species used for meat production

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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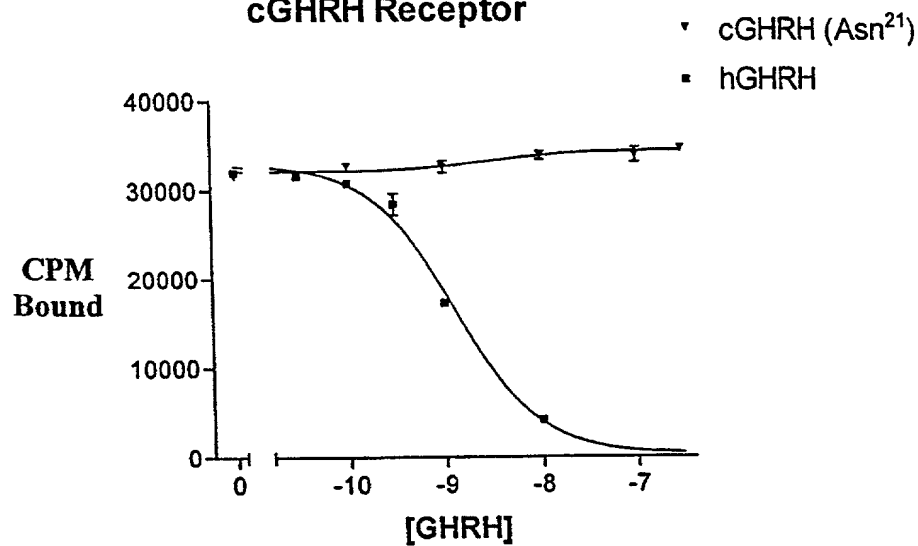
**Binding at Recombinant
cGHRH Receptor**

Fig. 1A

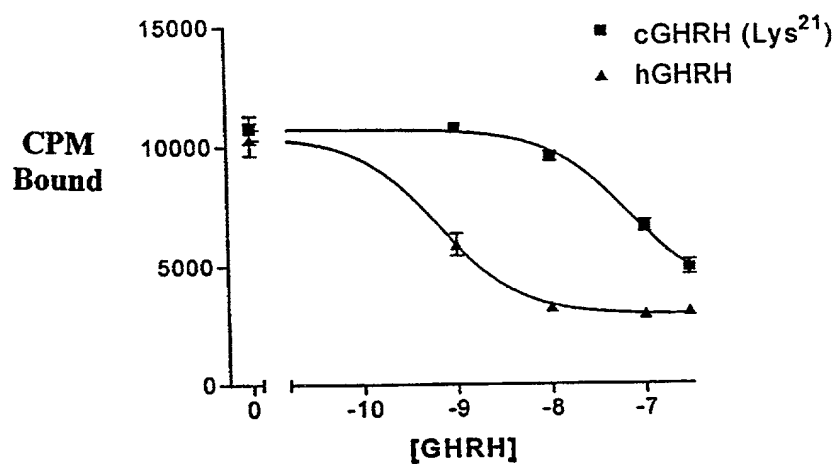
**Binding at Recombinant
cGHRH Receptor**

Fig. 1B

2/3

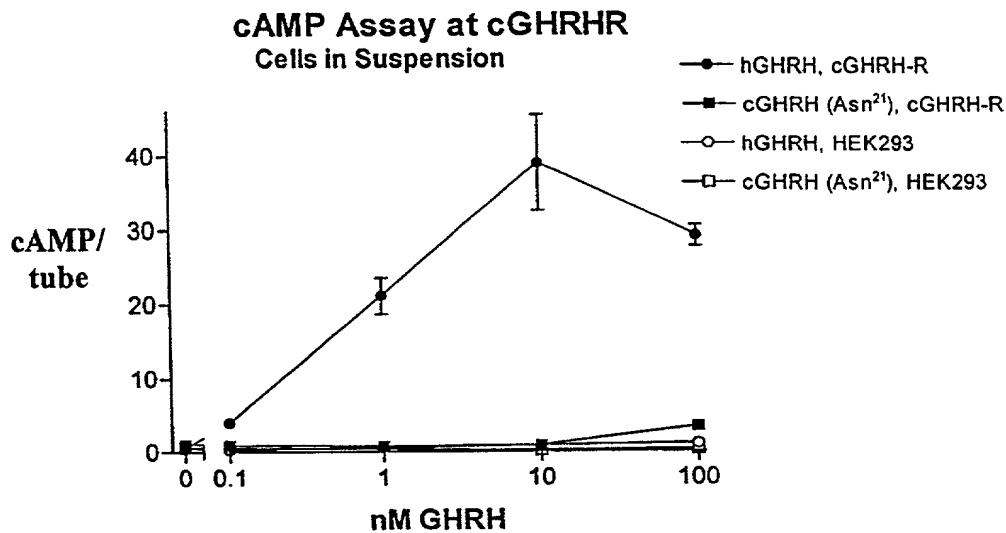


Fig. 2A

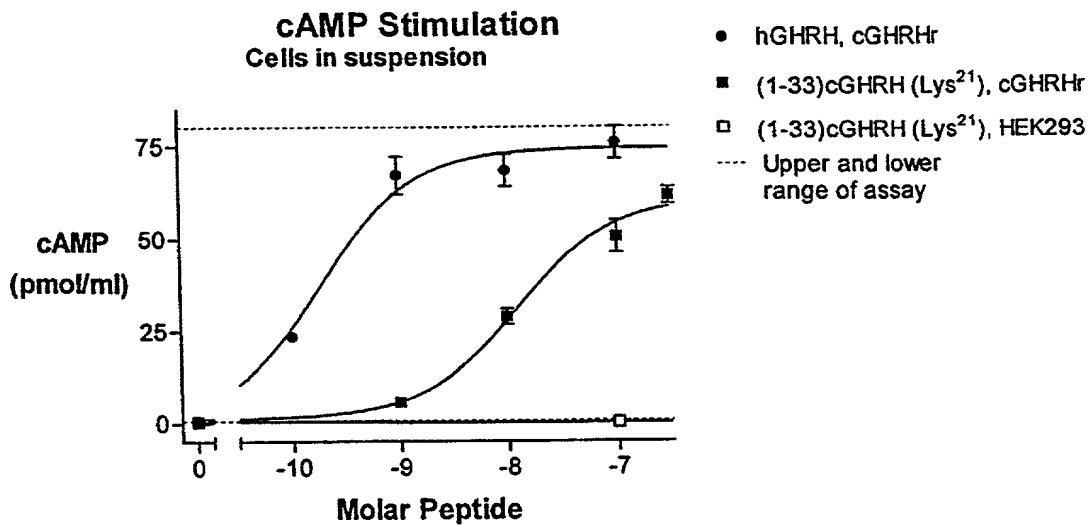


Fig. 2B

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Binding in Chicken Pituitary

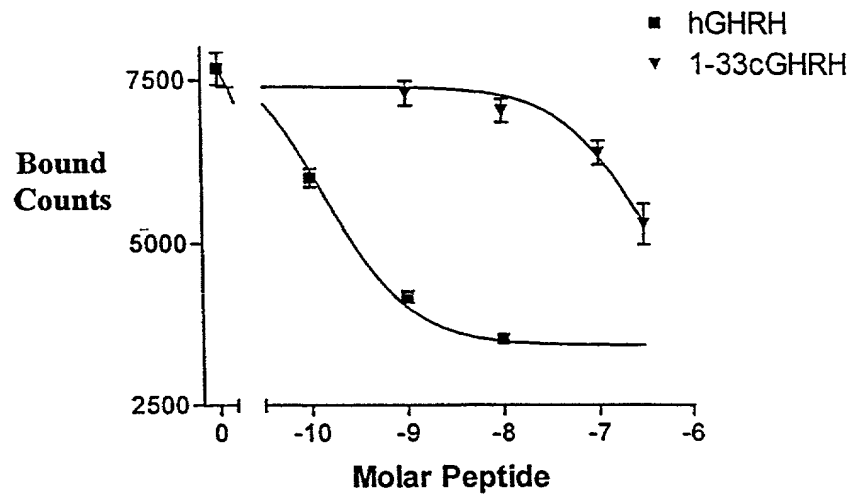


Fig. 3

033493-001

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As a below named inventor, I hereby declare that:

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I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHICKEN GROWTH HORMONE RELEASING HORMONE RECEPTOR

the specification of which (check only one item below):

- ☐ is attached hereto.
- ☐ was filed as United States application
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- ☒ was filed as PCT international application
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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

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COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119	
U.S.A.	60/138,768	12/06/1999	X Yes	No
U.S.A.	60/176,387	14/01/2000	X Yes	No
			Yes	No
			Yes	No
			Yes	No

(1001)

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Page 2 of 3

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Robert G. Mukai	28,531	Richard J. McGrath	29,195		
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Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SEVENTH INVENTOR, IF ANY	
Signature	

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Alexandria, Virginia 22313-1404

Address all telephone calls to: Brian P. O'Shaughnessy at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	Michael O. Thorner
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SECOND INVENTOR, IF ANY	Bruce David Gaylinn
Signature	
Date	
Residence (City, State, Country)	Louisa, Virginia, U.S.A.
Citizenship	
Mailing Address	16172 Louisa Road
City, State, ZIP, Country	Louisa, Virginia 23093, U.S.A.

033493-001

Attorney's Docket No.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHICKEN GROWTH HORMONE RELEASING HORMONE RECEPTOR

the specification of which (check only one item below):

is attached hereto.

was filed as United States application

Number

on

and was amended

on

(if applicable).

☒ was filed as PCT international application

Number US00/16135

on June 12, 2000

and was amended

on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119(a)-(d):

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119		
U.S.A.	60/138,768	12/06/1999	X	Yes	No
U.S.A.	60/176,387	14/01/2000	X	Yes	No
				Yes	No
				Yes	No
				Yes	No

Combined Declaration for Patent Application and Power of Attorney
 (Includes Reference to PCT International Applications)
 Attorney's Docket No. 033493-001
 Page 2 of 3

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Eric H. Weisblatt	30,505	Bruce T. Wieder	33,815
Robert S. Swecker	19,885	James W. Peterson	26,057	Todd R. Walters	34,040
Platon N. Mandros	22,124	Teresa Stanek Rea	30,427	Ronni S. Jillions	31,979
Benton S. Duffett, Jr	22,030	Robert E. Krebs	25,885	Harold R. Brown III	36,341
Norman H. Stepano	22,716	William C. Rowland	30,888	Allen R. Baum	36,086
Ronald L. Grudziecki	24,970	T. Gene Dillahunty	25,423	Brian P. O'Shaughnessy	32,747
Frederick G. Michaud, Jr.	26,003	Patrick C. Keane	32,858	Kenneth B. Leffler	36,075
Alan E. Kopecki	25,813	B. Jefferson Boggs, Jr.	32,344	Fred W. Hathaway	32,236
Regis L. Slutter	26,999	William H. Benz	25,952	Wendi L. Weinstein	34,456
Samuel C. Miller, III	27,360	Peter K. Skiff	31,917	Mary Ann Dillahunty	34,576
Robert G. Mukai	28,531	Richard J. McGrath	29,195		
George A. Hovanec, Jr.	28,223	Matthew L. Schneider	32,814		
James A. LaBarre	28,632	Michael G. Savage	32,596		
L. Joseph Gess	28,510	Gerald F. Swiss	30,113		
R. Danny Huntington	27,903	Charles F. Wieland III	33,096		



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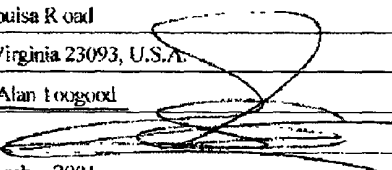
at (703) 836-6620.

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FULL NAME OF SOLE OR FIRST INVENTOR	Michael O. Thorne
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SECOND INVENTOR, IF ANY	Bruce David Gaylinn
Signature	
Date	
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Page 3 of 3

3-00

Citizenship	
Mailing Address	16172 Louisa Road
City, State, ZIP, Country	Louisa, Virginia 23093, U.S.A.
FULL NAME THIRD INVENTOR, IF ANY	Andrew Alan Toogood
Signature	
Date	11 th December 2001
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Citizenship	British
Mailing Address	10 Finbury Close
City, State, ZIP, Country	Olton, Solihull, West Midlands B92 8DH, UK
FULL NAME FOURTH INVENTOR, IF ANY	Steve Harvey
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	

☐ Additional inventors are being named on the Supplemental Additional Inventor(s) Sheet(s) attached hereto.

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(Includes Reference to PCT International Applications)**

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the specification of which (check only one item below):

- ☐ is attached hereto.
- ☐ was filed as United States application
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Frederick G. Michaud, Jr.	26,003	Patrick C. Keane	32,858	Kenneth B. Leffler	36,075
Alan E. Kopecki	25,813	B. Jefferson Boggs, Jr.	32,344	Fred W. Hathaway	32,236
Regis E. Slutter	26,999	William H. Benz	25,952	Wendi L. Weinstein	34,456
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George A. Hovanec, Jr.	28,223	Matthew L. Schneider	32,814		
James A. LaBarre	28,632	Michael G. Savage	32,596		
E. Joseph Gess	28,510	Gerald F. Swiss	30,113		
R. Danny Huntington	27,903	Charles F. Wieland III	33,096		



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Signature	
Date	
Residence (City, State, Country)	
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Mailing Address	
City, State, ZIP, Country	
FULL NAME SECOND INVENTOR, IF ANY	Bruce David Gaylinn
Signature	
Date	
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Citizenship	
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Date	
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Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SIXTH INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SEVENTH INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME EIGHTH INVENTOR, IF ANY	
Signature	
Date	
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WO 00/76455

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PCT/US00/16135

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